HIGH SENSITIVITY TUMOR CELL TRACKING IN VIVO USING QUANTUM DOTS

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Abstract

The sensitivity of optical imaging to quantum-dot labeled tumor cells was evaluated in an animal model. Tumor cells (SCC VII) were incubated with peptide-conjugated 705-nm emitting quantum dots (Quantum Dot Corp., Hayward, CA). Each mouse (nu/nu) was injected with a 50-µl aliquot of cell suspension subcutaneously into the left hind flank to simulate a small tumor. Mice were anesthetized and imaged (IVIS 200, Xenogen). Injections of 200,000 cells were detected through the skin, but smaller numbers were not. The results highlight the need for optimization of both the loading protocol and imaging parameters to enable detection of small tumors.

Introduction

An ultra-sensitive method for tracking subcutaneous cells in small animals may enable health researchers (*i*) to study progressive diseases such as cancer *in vivo* without the need for animal sacrifice; and (*ii*) to assess the performance of optical contrast-based imaging in relation to other cancer diagnostics currently used in the clinic. Laboratory-based methods for cell tracking involve laborious evaluation of tissue post-mortem, invasive *in vivo* microscopy techniques with limited fields of view [1, 2], or reporter genes, which may be difficult to translate to clinical use [3-5]. MicroPET and microSPECT represent non-invasive modalities for small animal *in vivo* imaging that have clinical counterparts. However, the methods suffer from dependence on radioactive materials, which are generally costly, pose a potential occupational hazard, and may be difficult to acquire or produce. Whole-body near-infrared (NIR) optical imaging is a non-invasive technique that offers great sensitivity to contrast agents *in vivo* without the use of radioactive materials [6]. Recent developments in the field of quantum dot (QD) contrast agents, which are brightly fluorescent nanocrystalline semiconductors, offer potential for enhancement of the sensitivity of NIR optical imaging [7, 8]. Therefore, the combination of QD contrast agents with NIR optical imaging may provide a sensitive method to track cells *in vivo* without resorting to radioactive agents and/or invasive techniques. We tested the feasibility of this approach by labeling tumor cells with a QD fluorescent indicator, injecting labeled cells at increasing numbers into mice, and then imaging the mice using a commercially available optical scanner.

Materials and Methods

Tumor cell line: The SCCVII tumor cell line was selected as it is commonly used for evaluation of new cancer treatments and can produce highly aggressive solid tumors when injected subcutaneously into mice (e.g. see [9]). Cells were grown in T75 flasks in Weymouth media containing 15% fetal calf serum and sodium bicarbonate under standard tissue culture conditions (37ºC with humidified atmosphere of 5% CO_2 with balance air). On the day of the experiment, the media from one flask was removed and replaced with 1 ml-0.25% trypsin. Detached cells were then suspended in 8 ml media and centrifuged at 300 x g for 5 min. The cell pellet was resuspended in 8 ml media and counted with the assistance of a hemocytometer. The volume of cell suspension was adjusted to achieve a cell density of 1 x 10^6 cells/ml.

Quantum dot labeling of cells: Labeling of tumor cells with quantum dots was performed with a Qtracker™ cell labeling kit (Quantum Dot Corp., Hayward, CA). A 40 nM stock solution of QD was prepared according to the manufacturer's protocol. A 500 µl aliquot of QD stock was added to 10 ml of cell suspension and incubated for 1 h at 37ºC. The cell suspension was centrifuged at 300 x g for 5 min and the supernatant was replaced with 10 ml media. This step was repeated twice more, except upon the final resuspension, 2 ml media was used. Cells were counted and media was added to achieve 4 x 10^6 cells/ml. A dilution series was prepared from this final stock solution of cells in a fluorescence compatible 96-well plate. Each well contained 200 µl of cell suspension. Wells were prepared in triplicate at the following cell densities: 4 x 10^6, 4 x 10^5, 4 x 10^4, 4 x 10^3 and 4 x 10^2 cells/ml.

Optical imaging of the plate: The 96-well plate was optically imaged (IVIS 200, Xenogen Corp., CA) at 37ºC. Optical images were collected using predefined filter settings: excitation used a filter optimized for the organic dye DsRed (~500-

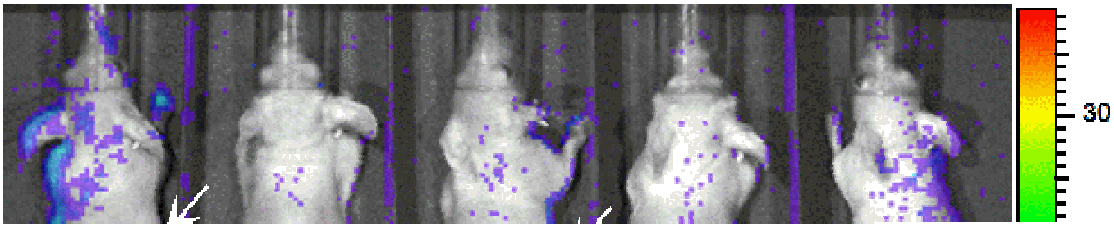
550 nm), and emission was collected with a filter optimized for the fluorophore Cy5.5 (~695-770 nm). Exposure time was 1 s at an aperture of f/2. The photon count from each well was determined with ROI analysis built into the IVIS imaging software.

In vivo optical imaging: Male nude (nu/nu) mice 7–8 weeks old were bred and housed under defined flora and specific pathogen-free conditions in the Stanford Research Animal Facility of the Department of Comparative Medicine. All animal experiments were approved by and complied with the regulations of the Stanford University Care Panel. Five mice (nu/nu) were induced in an isoflurane atmosphere. The use of nude mice simplifies interpretation of the optical images. The mice were then transferred to the stage of an optical imager (IVIS 200, Xenogen Corp., CA) and kept anesthetized by isoflurane inhalation via five nose cones. Optical images were collected using the filter and aperture settings described above, except the exposure was 10 s. After acquisition of a baseline image of the five mice, a 50 µl aliquot of cell suspension was injected subcutaneously into the left hind flank of each mouse. Each injection contained cells at a density that corresponded to a value given above.

Results

When the QD contrast agent was loaded into SCCVII cells, the fluorescence intensity recorded from plated cells increased above background levels (~ 40 x 10^6 photon counts/s/sr/cm^2) for wells at a cell density of 400 cells/µl or more (Fig. 1). For cells injected into mice, the fluorescence emission from the subcutaneous bleb that contained 200,000 cells was detectable above background. However, the fluorescence emission from blebs with fewer numbers of cells could not be detected.

Fig. 1. Fluorescence detection of QD in cultured tumor cells. Radiance from SCCVII tumor cells loaded with 705-nm emitting QD extracted from fluorescence images of a 96 well plate by regions of interest (ROI). Wells were prepared in triplicate and contained SCCVII cells at one of five cell densities in 200 µl Weymouth media. Prior to imaging, cells were loaded at 10^6 cells/ml with 705 nm-emitting QD (2 nM) for 1 h at 37ºC. Symbols represent the average radiance measured from three ROI. Error bars represent standard errors but are smaller than the symbol size in all cases. Images were obtained with a Xenogen IVIS 200 at f/2 with a 1 s exposure using an excitation filter optimized for DsRed (~500-550 nm) and an emission filter optimized for Cy5.5 (~695-770 nm).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autofluorescence and light leakage. These improvements should permit the detection of clusters of QD-loaded cells below the skin of a small animal at a threshold below 200,000 cells.

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References

1. Voura, E.B., J.K. Jaiswal, H. Mattoussi, and S.M. Simon, *Tracking metastatic tumor cell extravasation with quantum dot nanocrystals and fluorescence emission-scanning microscopy*. Nat. Med., **10**, 993-8, (2004).

2. Chambers, A.F., A.C. Groom, and I.C. MacDonald, *Dissemination and growth of cancer cells in metastatic sites*. Nat. Rev. Cancer, **2**, 563-72, (2002).

3. Contag, P.R., I.N. Olomu, D.K. Stevenson, and C.H. Contag, *Bioluminescent indicators in living mammals*. Nat. Med., **4**, 245-7, (1998).

4. Hoffman, R.M., *In vivo imaging of metastatic cancer with fluorescent proteins*. Cell Death Diff., **9**, 786-789, (2002).

5. Chishima, T., Y. Miyagi, X. Wang, H. Yamaoka, H. Shimada, A.R. Moossa, and R.M. Hoffman, *Cancer invasion and micrometastasis visualized in live tissue by green fluorescent protein expression*. Cancer Res., **57**, 2042-7, (1997).

6. Benaron, D.A., *The future of cancer imaging*. Cancer Metastasis Rev., **21**, 45-78, (2002).

7. Green, M., *Semiconductor Quantum Dots as Biological Imaging Agents*. Angew. Chem. Int. Ed., **43**, 2-4, (2004).

8. Becker, A., C. Hessenius, K. Licha, B. Ebert, U. Sukowski, W. Semmler, B. Wiedenmann, and C. Groetzinger, *Receptor-targeted optical imaging of tumors with near-infrared fluorescent ligands*. Nat. Biotech., **19**, 327-331, (2001).

9. Maxim, P.G., J.J.L. Carson, S. Ning, S.J. Knox, A.L. Boyer, C.P. Hsu, D.A. Benaron, and J. Walleczek, *Enhanced Effectiveness of Radiochemotherapy with Tirapazamine by Local Application of Electric Pulses to Tumors*. Rad. Res., **162**, 185-193, (2004).

10. Chan, W.C.W. and S. Nie, *Quantum Dot Bioconjugates for Ultrasensitive Nonisotopic Detection*. Science, **281**, 2016-2018, (1998).